ROS detection kit

Catalogue No.	Product Name	Size
K050	ROS detection kit	>100test

Introduction

The ROS detection kit (Reactive Oxygen Species Assay Kit) is a kind of kit for active oxygen detection using fluorescent probe DCFH-DA. DCFH-DA itself has no fluorescence, can freely pass through the cell membrane into the cell, then it can be hydrolyzed by intracellular esterases to DCFH. DCFH can not penetrate membrane, so the probe is very easy to be loaded into the cell. Intracellular ROS can oxidize DCFH without fluorescence to fluorescent DCF. We can know intracellular ROS level by detecting fluorescence of DCF.

This kit provides reagents Rosup as positive control for ROS detection. Rosup is compound mixture, the concentration of 50mg/ml.

The kit has low background, high sensitivity, wide linear range, easy to use.

This kit can be used to test 100-500 samples.

Kit Components

Item	Size
DCFH-DA (10mM)	0.1ml
Reactive Oxygen positive control (Rosup, 50mg/ml)	1ml
Product Description	1copy

Storage and Stability

All reagents are shipped on dry ice. Upon receipt, the kit should be stored at -20°C or -80°C for one year.

Avoid repeated freezing and thawing.

Precautions for Use

The residual probes not enter the cells must be washed thoroughly, otherwise it will lead to high background.

After loading the probes and washing residual, scanning of the excitation wavelength and scanning of the emission wavelength can be performed to confirm whether the probe is in good condition of loading. For the excitation and emission spectra of DCF, please refer to the next page.

Try to shorten the time from loading to determine (except to the stimulation time) to avoid possible errors.

This product is only limited to scientific research personnel, can not be used for clinical diagnosis or treatment, food or medicine, must not be stored in the ordinary residential.

For your safety and health, please wear clothes and wear disposable gloves.

Assay Procedure

1. Loading probe

For cells with short stimulation time(within 2 hours), first load probes ,then stimulate cells with Reactive Oxygen positive control or interested drugs. For cells with long stimulation time (more than 6 hours), first stimulate cells with Reactive Oxygen positive control or interested drugs, then load probes.

In situ loading probe: This method applies only to adherent cells. Dilute DCFH-DA with serum-free culture medium according to 1:1000, the final concentration is 10 μmol / L. Remove cell culture， add appropriate volume of diluted DCFH-DA. Added volume should fully cover the cell， usually for a hole of six hole plate, not less than 1 ml of diluted DCFH-DA should be added. Incubate at 37 °C for 20min. Then wash three times with serum-free cell culture to completely remove extracellular DCFH-DA. Usually after ROS positive control stimulate cells for 20-30 minutes, the level of reactive oxygen species can be significantly improved.

Collect cells loading probe: Dilute DCFH-DA with serum-free culture medium according to 1:1000, the final concentration is 10 μmol / L. Cells were collected and then suspended in diluted DCFH-DA with final concentration of one million to twenty million / ml. Incubate at 37 °C for 20min, mix up and down every 3-5min. Wash three times with serum-free cell culture to completely remove extracellular DCFH-DA. Stimulate cells with Reactive Oxygen positive control or interested drugs or stimulate after dividing cells into several parts. Usually after ROS positive control stimulate cells for 20-30 minutes, the level of reactive oxygen species can be significantly improved.

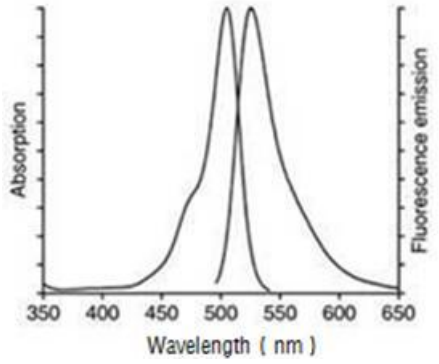
Note: only join Rosup in the positive control hole as a positive control, the remaining holes need not added.

2. Detection

In situ loading probe samples can be directly observed by laser confocal microscopy, or observed by fluorescence spectrophotometer, fluorescence microplate or flow cytometry after collecting cells. Collect cells loading probe samples can be observed using fluorescence spectrophotometer, fluorescence microplate or flow cytometry or directly observed by laser confocal microscopy.

3. Parameter setting

Use 488nm excitation wavelength, 525nm emission wavelength, detect the intensity of fluorescence before and after stimulation in real time or by time points. The fluorescence spectra of DCF are very similar to those of FITC, can use parameters of FITC to detect DCF. The excitation spectra and emission spectra of DCF refer to the diagram.



4. Other instructions

Positive control can be used according to the ratio of 1:1000. For example, add 1 μl positive control to

1 ml loaded probe cells. You can observe the reactive oxygen levels significantly elevated after stimulation for 20-30 minutes. For different cells, reactive oxygen positive control effect may have a greater difference. If the increase of reactive oxygen species is not observed within 30 minutes after stimulation, the concentration of active oxygen positive control can be appropriately increased. If reactive oxygen species rise too rapidly, the concentration of active oxygen positive control can be reduced appropriately.

In addition, for some cells, if the negative control cells without stimulation also have relatively strong fluorescence, you can dilute DCFH-DA according to the ratio of 1:2000-1:5000, final DCFH-DA concentration was 2-5 μ mol / L. Probe loading time can be adjusted according to the situation properly within 15-60 minutes. The positive control of reactive oxygen species (Rosup) only used as positive control samples, it is not necessary to add active oxygen positive control in each sample.